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Towards understanding the abundance of non-pollen palynomorphs: A comparison of fossil algae, algal pigments and *seda*DNA from temperate lake sediments

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Abstract

Given the increased interest in non-pollen palynomorphs (microscopic objects other than pollen identified from the pollen slides) in palaeoecological studies, it is necessary to seek a deeper understanding how reliable the obtained results are. By combining quantitative information of algal pigments and phylotaxonomical resolution from sedimentary ancient DNA (*seda*DNA), we validate the richness and abundance of aquatic non-pollen palynomorphs – fossil algae, in the sediment of a small temperate lake. For the first time, fossil and *seda*DNA algae data were combined in a composite data-set and algal turnover rates in time were reconstructed for the last 14,500 years. This comparison will serve as an indication to what extent fossil algae can be used to answer

different research questions and to reveal if it is reliable to base palaeoecological interpretation solely on fossil algae identified from the pollen slides.

Keywords: non-pollen palynomorphs; palaeopigments; *seda*DNA; community richness

1. Introduction

In addition to pollen identification, palynologists have recently started to consider the value of other microscopic objects (e.g. fungi, algae, plant and animal remains) from pollen slides, referred to as *sensu stricto* non-pollen palynomorphs (NPP). NPP are found in various environments such as in sediments underlining their potential in palaeoecological studies (Shumilovskikh et al., 2016; Lenarczyk et al., 2015; Aptroot and van Geel, 2006; Medeanic, 2006; Turner et al., 2014; Demske et al., 2013). Whilst there are studies utilizing NPP in addressing significant research questions, such as evaluating ecological impacts of the late Quaternary megaherbivore palaeodiet and extinctions (Gill, 2014; van Geel et al., 2011), revealing fungi influence on forest dynamics (Latałowa et al., 2013), and estimating biotic turnover rates during the Pleistocene-Holocene transition (Stivrins et al., 2016), only a handful of studies deals with the comparison of NPP with other proxies and the validation of NPP as a palaeobiological metric (Etienne and Jouffroy-Bapicot, 2014; Gill et al., 2013; Wood and Wilmshurst, 2013). Given the increased interest in NPP, it is necessary to seek a deeper understanding how reliable metric it is in quantifying temporal changes in ecosystems.

Based on Miola's (2012) review, there are more than 1300 NPP descriptions available up today and the list of NPPs is still growing. Palaeoecologists are aware that there may be certain limitations in NPP – for example, part of NPP do not preserve as fossils. Selective preservation and thus, distribution, is an issue for most palaeoecological analyses. Therefore, the results must be

interpreted very carefully. Nevertheless, as all biological organisms prefer certain environmental conditions, even scattered information about NPP in time and space can aid greatly the palaeoecological reconstructions.

NPPs are formed both in terrestrial and aquatic environments and the bulk of them are characteristic of the local environment, they thus do not travel long distances from their source of origin (van Geel, 2001). Although, NPP identified alongside pollen analysis has been proven useful in understanding of the changes in lakes in the past (van Geel, 2001; van Geel et al., 1994; Jankovská and Komárek, 2000), majority of the studies have focused on paludified or terrestrial environments (Dietre et al., 2017; Shumilovskikh et al., 2015; Chmura et al., 2006) leaving less attention to aquatic environment. No matter whether NPP is discovered from terrestrial or aquatic sediments, question remains – how reliable the results are?

Fortunately, regarding aquatic NPP (fossil algae) identified from gyttja, there are useful recent methods to evaluate NPP performance, namely algal pigments and sedimentary ancient DNA (*seda*DNA). Algae contain pigments (lipid soluble chlorophylls and carotenoids), which are in most cases well preserved in lake sediments (Leavitt and Hodgson, 2001). Hence, pigments can be used to reconstruct the past quantitative phytoplankton community dynamics (Reuss et al., 2010; Leavitt and Hodgson, 2001; Fietz et al., 2007; Tonno et al., 2013; Deshpande et al., 2014). Although algal pigments provide quantitative estimations about the major phytoplankton groups, they are limited in indicating the abundance of lower taxonomic groups such as genera or species (Leavitt and Hodgson, 2001).

Sequencing of environmental DNA, specifically *seda*DNA, from lake sediments have recently become more available that offers identification of specific palaeo communities and groups of

organisms (Willerslev et al., 2003; Graham et al., 2016; Coolen et al., 2013). There are two significant differences between *seda*DNA and NPP. *seda*DNA can reconstruct species belonging to all domains of Life: Eukaryota, Bacteria and Archaea, thus comprising in magnitudes higher phylogenetic diversity than NPP, which due to taphonomical issues represents only selective diversity of species. On the other hand, NPP usually covers representatives from two domains e.g. Bacteria: cyanobacteria and Eukaryota: green algae, whilst *seda*DNA requires a complex targeted-methodology/sequencing to represent more than one domain. In the current study, we compared *seda*DNA and NPP algae from Eukaryota, which are one of the most abundant microscopic remains from lacustrine palynological samples (Jankovská and Komárek, 2000; Stivrins et al., 2015; Wacnik, 2009; Sarmaja-Korjonen et al., 2006). Since the domain of Bacteria was not targeted by *seda*DNA, the comparison between NPP cyanobacteria and *seda*DNA bacteria should be done and discussed elsewhere. Furthermore, by combining the semi-quantitative information from algal pigments and higher taxonomical resolution from *seda*DNA, it is now possible to validate the abundance of NPP in lacustrine environments.

Here, we explore whether the amount of fossil algae (aquatic NPP) identified alongside pollen analysis correlate with semi-quantitative and qualitative values of algal pigments and *seda*DNA. This evaluation will serve as an indication to what extent fossil algae can be used to answer different palaeoecological research questions and is it sound to rely the interpretation solely on fossil algae discovered from the pollen slides. In the current study, the term ‘fossil algae’ is used to indicate NPP phytoplankton identified from the pollen slides.

2. Material and methods

2.1. Study area, sampling and chronology

Studied lake Lielais Svētiņu (mean water depth 2.9 m; maximum depth 4.9 m; area 18.8 ha) is located in Latvia, eastern Baltic. The lake is a mesotrophic-dystrophic type drainage lake with a relatively moderate and late human impact (Stivrins 2014; 2015). The present-day topography was formed during the Weichselian glaciation and deglaciation (Zelčs and Markots, 2004). The bedrock consists of Devonian dolomite covered by Quaternary deposits. The catchment area of 12 km^2 is predominantly forested and partly covered by agricultural fields. The climate in the area is a combination of continental and maritime influences, with mean annual temperature of +5.2 °C, mean July temperature of +16.9 °C, and mean December temperature –4.1 °C (Stivrins et al., 2014).

Fig.1. Location of the studied Lake Lielais Svētiņu.

Lake Lielais Svētiņu was sampled in March 2009, and 2013 using a multiple parallel overlapping sediment cores from ice using a Russian type corer with a diameter of 10 cm. The sediment thickness reached to 1535 cm that comprise continual sediment record covering the last 14,500 years. The chronology of the core retrieved in 2009 is based on 20 radiocarbon dates (Stivrins et al., 2015). Age-depth model was built using the OxCal 4.2.4 deposition model (Bronk Ramsey, 2009) and the IntCal13 calibration set (Reimer et al., 2013). The sediment core used for algae pigments and *seda*DNA analyses was correlated with the year 2009 core according to the changes in lithology and loss-on-ignition, enabling to adjust Stivrins et al. (2015) age-depth model using about 30

correlation levels (Kisand et al., under review). All calibrated ages in the text refer to years before the present (cal. BP=AD 1950).

2.2. Fossil algae, algal pigments and *seda*DNA

Fossil algae were recovered from the core obtained 2009 and have been published in Stivrins et al. (2015). Shortly, subsamples for microfossil analysis were prepared and analyzed along with pollen analysis. Altogether 101 samples of known volume were treated using standard pollen preparation method (10% HCl, 10% KOH and acetolyzed for 3 min). *Lycopodium* spores were added to estimate phytoplankton accumulation rates. Commonly, the relative abundance of NPP is expressed in percentages that are estimated against the sum of counted pollen. In the current study, relative proportion (percentages) of phytoplankton is based on 1) the sums of phytoplankton and 2) the sums of phytoplankton enabling comparison between these two approaches.

Palaeopigment subsamples were obtained from 2013 core with the resolution of one sample after every 5 cm. Analysis of collected 93 palaeopigment subsamples followed the recommendations of Leavitt and Hodgson (2001). Briefly, sediment samples were first freeze-dried and marked by internal standard, thereafter sedimentary pigments were extracted with the mixture of acetone and methanol (80:20 v:v) at -20 °C in the dark for 24 h. Finally, extracts were clarified before chromatographic analysis by filtration through a 0.45 µm filter (Millex LCR, Millipore) to remove any particles.

Reversed–phase high–performance liquid chromatography (RP-HPLC) was applied to separate the pigments. Shimadzu Prominence (Japan) series binary gradient system with a photodiode array (PDA) and fluorescence detectors was used (see Tamm et al. 2015 for more details). Peak identification and quantification was made by commercially available external standards from DHI (Denmark).

144 In the present study, it was assumed that markerpigments Fucoxanthin, Diadinoxanthin and
145 Diatoxanthin represent diatoms (Desphande et al 2014; McTigue et al 2015), while pigments
146 Zeaxanthin, Canthaxanthin and Echinenone were selected to represent cyanobacteria (Roy et al.
147 2011). Lutein and Chlorophyll *b* tracked green algae dynamics, although these pigments are also
148 present in higher plants (Waters et al. 2013). Alloxanthin and *a*–Carotene was analysed to identify
149 the dynamics of Cryptophytes, while Peridinin indicated the abundance of Dinophytes (Leavitt and
150 Hodgson 2001).
151
152 *seda*DNA was extracted using PowerSoil® DNA Isolation Kit (MoBio) from sediment samples (0.3
153 g wet sample) in three biological replicates. DNA extraction was performed under positive-flow
154 hood (Kojair K-safety KR-125) exposed to UV light prior to extractions, surfaces were cleaned with
155 Thermo Scientific™ DNA AWAY™ Surface Decontaminant. Raw SE reads were quality trimmed
156 deleting all nucleotides below Q30 (Trimmomatic V0.32), paired and clustered (similarity threshold
157 of 97%) using CD-HIT-OUT to obtain molecular operational taxonomic units (mOTUs). Universal
158 18S rRNA primer pair covering V4 region (Tedersoo et al., 2015) was used to match the length of
159 fragment suitable for Illumina PE250 sequencing (<450-460 bp). Cluster sequences were aligned
160 and phylotaxonomy was determined using SINA aligner (Pruesse et al., 2012) and SILVA ssuRNA
161 database version 115 for 18S rDNA sequences (Quast et al., 2013; Pfeiffer et al., 2014). Clusters
162 with different domain affiliation (mostly Bacteria) and clusters with low number of reads (<4 reads)
163 were removed from further downstream analysis. More details on sedaDNA methodology see
164 Kisand et al. (in press). More details on *seda*DNA methodology see Kisand et al. (in press).
165
166 2.3. Data analysis
167 As a whole, algal remains can be considered both as binary and quantitative data, but similarly to
168 terrestrial plant macrofossils and pollen, fossil algae records from the sediment samples do not

reflect the entire taxonomic diversity (Stivrins et al., 2016). Nevertheless, algae from small to medium sized lakes, such as from Lielais Svētiņu, indicate at least partly the spatiotemporal variation in local in-lake productivity and population abundances. Algal data from *seda*DNA is currently available as binary data that provide information on taxon presence in the lake. Note that taxon absence from *seda*DNA can be related to various other aspects, which are mostly caused by low resolution of 18S rDNA sequences at genus and species level and is not necessarily reflecting the true absence of species.

Considering their local source, we treated both fossil and *seda*DNA algae as binary data and pooled them into one composite algae dataset to obtain a more complete and reliable record of the algal species richness. By doing so, for the first time, we demonstrate how fossil algae (Bacteria: cyanobacteria and Eukaryota: green algae) counted from the pollen slides can be integrated with *seda*DNA (Eukaryota: green algae), thus introducing a new opportunity to solve future palaeoecological questions, such as changes in biodiversity due to climate change. As an example, we run Sørensen dissimilarity index that provides algal temporal turnover estimates (beta diversity of species assemblages in time) for the last 14,500 years. Along with the Jaccard index, Sørensen index is one of the most widely used dissimilarity indices and is regarded as one of the most effective presence/absence measures (Magurran, 2004). Sørensen dissimilarity index is calculated by $\beta_{SOR} = 1–(2a/(2a+b+c))$, where 'a' indicates the total number of species present in both samples, 'b' refers to the number of species present only in sample one and 'c' to the number of species present only in sample two. The index ranges from zero to one, where zero indicates that the communities have identical species composition while one indicates that two communities have no shared species and thus full turnover.

3. Results

Relative proportions (percentages) of fossil algae vary significantly if they are estimated based on sum of algae or pollen (Fig. 2 a,b). Pollen based proportions correlate with fossil algae accumulation rates (Fig. 2 c) and can be an artefact due to used equation. However, proportions of fossil algae based on algae sum, solely indicates changes within the algal population.

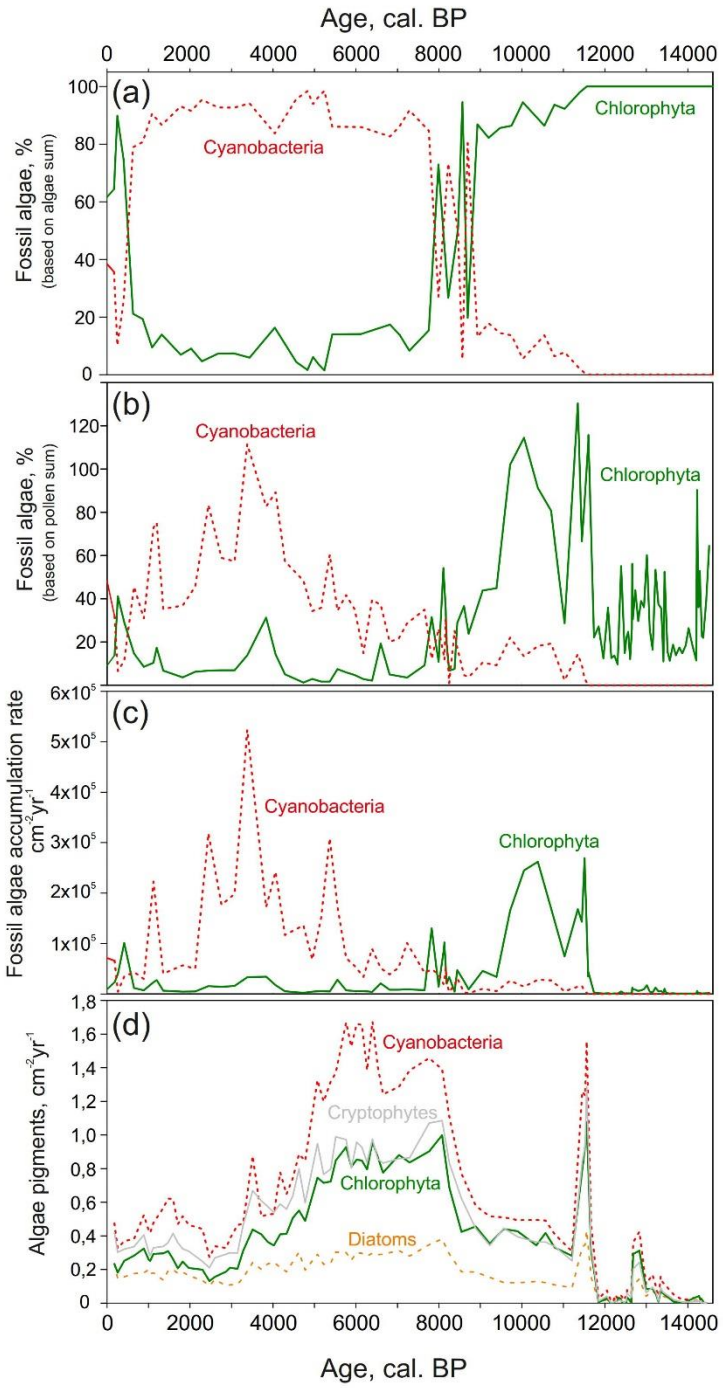


Fig. 2. Comparison of (a) fossil algae based on algal sum, %, (b) fossil algae based on pollen sum, %, (c) fossil algae accumulation rate, $cm^{-2}yr^{-1}$, and (d) accumulation rates of algae pigments, $cm^{-2}yr^{-1}$.

Phytoplankton accumulation rates (Fig. 2 c) were the lowest during the Lateglacial (14,500–11,700 cal. BP). Chlorophyta dominated in early Holocene (11,700–9000 cal. BP), after which their values were low throughout the Holocene. Since 7500 cal. BP cyanobacteria accumulation rates increased with a maximum from 4500 to 2500 cal. BP, whereas earlier their accumulation rates were insignificant (no accumulation during the Late glacial).

Although five major algal pigment groups were retrieved, only four of them (diatoms, cyanobacteria, chlorophytes, cryptophytes) can be considered as dominant (dinophytes were excluded due to minor values). Period from 14,500 to 11,700 cal. BP was characterized by low pigment accumulation, and the highest accumulation occurred from 8200 to 5000 cal. BP (Fig. 2 d).

For the first time, the fossil phytoplankton (including also Bacteria: cyanobacteria) and *seda*DNA algae data were combined into one composite diagram (Fig. 3) that comprises as many as 87 taxa. The highest numbers of phyla, order and species were observed for *seda*DNA (Fig. 4). Only minor share of these were overlapping with the fossil algae.

Sørensen dissimilarity index indicated overall lower values for the Pleistocene, i.e. Lateglacial (14,500–11,700 cal. BP) and early/middle Holocene (8400–7900 and 6300–4700 cal. BP) (Fig. 5) indicating lower biotic turnover in time. The highest sample dissimilarities and thus highest turnover rates were observed for early (11,700–8500 cal. BP), middle (7800–6300 cal. BP) and late Holocene (4700 cal. BP–present).

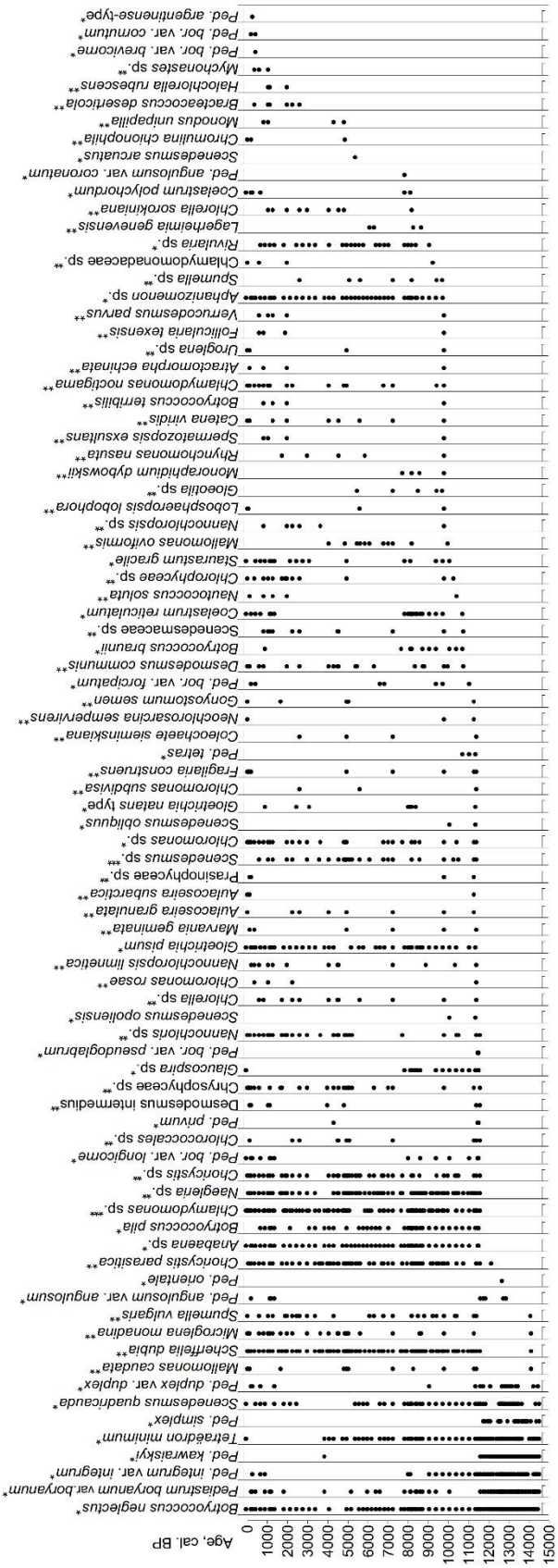


Fig. 3. Composite diagram based on fossil algae (NPP) and algal *seda*DNA data, comprising 87 taxa from 14,500 cal. BP to present. Fossil algae – *, *seda*DNA – **, overlapping – ***.

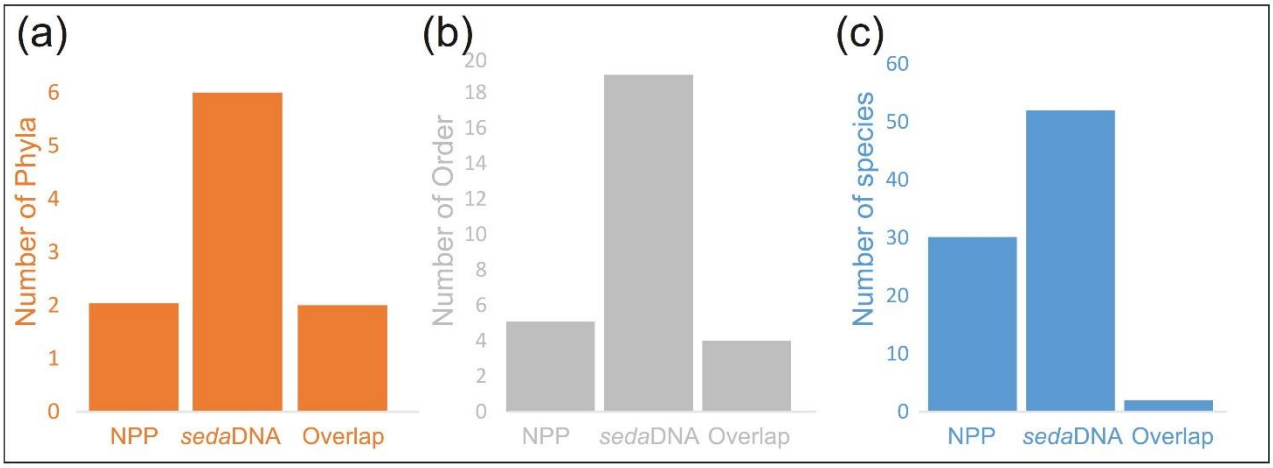


Fig. 4. Comparison of the taxonomical level (a) phyla, (b) order and (c) species identified by NPP (fossil algal remains) and *seda*DNA, and their overlap. NPP cyanobacteria were excluded from this comparison.

4. Discussion

4.1.Fossil algae accumulation rates are not direct reflection of biomass

For fossil algae representation, researchers typically use percentages instead of concentration, and even more seldomly, accumulation rates. Percentages are commonly estimated against pollen sum that by default is a biased way to proceed, if NPP are not pollen. There are methods how to estimate microscopic object concentration per sample (volume) and even per year (Stockmarr, 1971). Recently, Wood and Wilmshurst (2013) showed that the interpretation based on percentages might lead to incorrect interpretations of expansion or extinction events in sedimentary records. Therefore, the accumulation rates of NPP can be a reasonable way to overcome this issue. However, as we demonstrate here (see Fig. 2), estimated accumulation rates of fossil algae show a different pattern compared with algal pigments. The reason is most likely linked to at least two factorsand study design: 1) natural processes such as decomposition and preservation of algae and 2) pollen sample

chemical treatment. Next, we will discuss these factors in detail and the groups "seen" in pigments versus NPPs (fossils need to have recognizable morphology as is discussed below).

Haselwander and Oboh-Ikuenobe (2017) study on algal preservation in shallow freshwater lakes in Missouri, USA, demonstrated that *Staurastrum* sp., *Botryococcus* sp., *Pediastrum simplex* var. *pseudoglabrum*, *P. integrum* and *P. boryanum* var. *pseudoglabrum* preserve well in lake sediments. In contrast, species such as *Sphaeorocystis* and *Ceratium hirundinella* do not preserve well. These findings are in line with other studies indicating that *Scenedesmus* sp., *Tetraëdron* sp., *Pediastrum* sp., *Coelastrum* sp., *Staurastrum* sp. and cyanobacteria (e.g. *Anabaena* sp., *Aphanizomenon* sp. and *Gloeotrichia* sp.) are the most common fossil algae remains in the samples because their cell wall material contains compounds, which confer resistance to bacterial decay (Bellinger and Sigee, 2010; Fey et al., 2010; Jankovská and Komárek, 2000; Weckström et al., 2010). Therefore, reconstructed algal population and accumulation dynamics are biased *per se*, leading to overrepresentation of some taxa and underrepresenting the other.

Pigments stored in sediment have different chemical stability and preservation (Leavitt and Hodgson 2001). However, in upper sediment layers, which are not consolidated, the degradation of pigments is highest to compare with historical sediment layers (Tõnno et al. 2013). Microbial activity and thus degradation processes of settled material in upper sediment layers are much more intensive that in deeper (historical) sediments (Wetzel 2001).

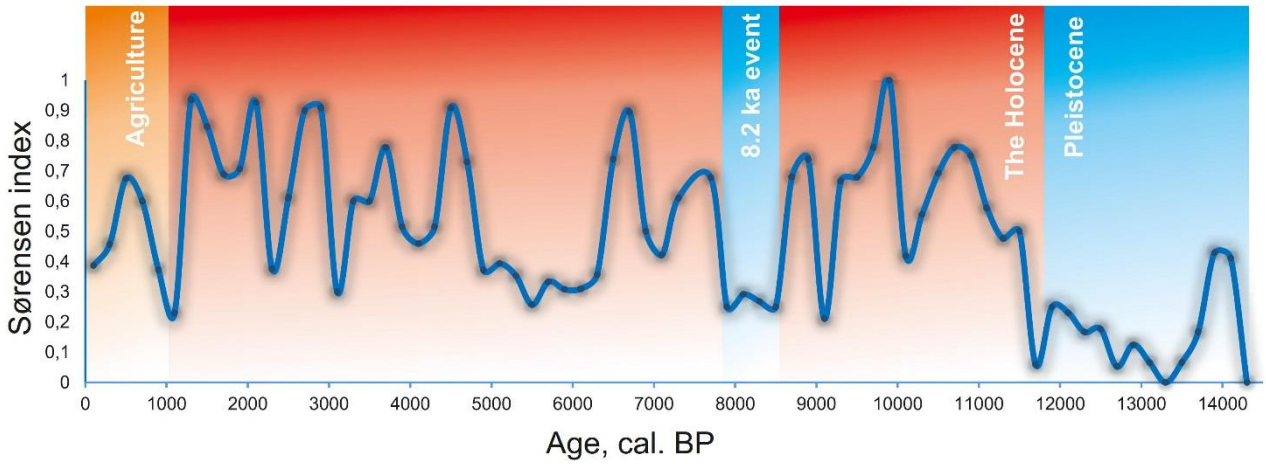
Another important aspect is palynological preparation that includes several steps of chemical usage – hence all NPP usually undergo the same treatment (Chambers et al., 2011). Riddick et al. (2017) demonstrated that the application of acetolysis, an oxidizing technique common in palynological preparation, significantly destructs dinoflagellate cysts. Even more significantly, it decreases the

abundance of desmids (green algae) with a reported mean 87% decrease. While observations of destructive effect of acetolysis has been explored also for other wide group of NPP – coprophilous fungi spores (van Asperen et al., 2016), the application of acetolysis shown to be useful in phytoliths extraction (da Costa et al., 2016). We agree with previous suggestions (Riddick et al., 2017; van Asperen et al., 2016) that step of acetolysis should be of limited use, if not excluded at all, in a future fossil algae identifications. If fossil algae are the main study subject rather than a side-project within a pollen routine work, counting NPP from smear slides seem to be the best option.

4.2. Taxonomic richness

As expected, our results demonstrate higher phylotaxonomic richness for the *seda*DNA than for the taxonomic richness of fossil algae. Small overlap at species level is not surprising due to low resolution of 18S rDNA sequences in Eukaryotes in general. In addition, NPP sample preparation includes sediment treatment using 10% KOH (potassium hydroxide) that dissolves all siliceous material including diatoms, not to mention that diatom analysis have a different preparation method. Hence, these are presented only in *seda*DNA, and overall biomass showed by algae pigments (Fig. 2). Only bacteria and chemically resistant algae survive the whole cycle from their production in the lake, through sedimentation to pollen sample preparation, identified under the microscope. In contrast, *seda*DNA and pigments identify even those algal species that have long lost their cells and left their fingerprint at molecular level. Although it would seem that solely *seda*DNA can be used to reconstruct past algal diversity, unfortunately, it has its merits. For instance, to obtain information of species, it is necessary to use higher resolution regions in genomes suitable for species detection, for example ITS, also to have reference barcodes in genebanks against which to compare obtained record. Methodology and process itself might be time consuming, and in most cases, also expensive. In addition, taxon can be detected only if their molecular chains are not fragmented and

295 are long enough to detect by DNA sequencing. In current study, we did not analyse Bacteria
296 (including cyanobacteria) in *seda*DNA and for *Pediastrum* resolution power in Archaeplastida was
297 relatively low. Probably mentioned circumstances of NPP and *seda*DNA methods could be a reason
298 why only a few phyla, order and species were overlapping. Since the domain of Bacteria was not
299 targeted in *seda*DNA, the comparison between NPP cyanobacteria and *seda*DNA Bacteria should
300 be done and discussed elsewhere. Collectively, although each method has their pros and cons, they
301 both complement each other, as highlighted by the composite data-set we display (Fig. 3, 5) and
302 discuss further.
303
304 *4.3. Implications and future prospect of non-pollen palynomorphs*
305 There are several take-home messages from the current study that are implacable for future NPP
306 analyses. We underline that it is worth to keep tracking NPP alongside routine pollen analysis as it
307 gives additional insights for various ecological aspects (natural and anthropogenic). Combination of
308 fossil algae and *seda*DNA record complete taxonomic richness and can be used to reconstruct for
309 instance functional groups or biodiversity through time. Our results show that fossil algae increased
310 overall algal diversity of phyla, orders and species. Sure enough, solely algal pigments of
311 cyanobacteria can be used to reconstruct their abundance, but that gives only quantitative biomass
312 estimates, while fossil algae provide partly from both – biomass and taxonomy. On the other hand,
313 algal pigments are the most quantitative, the *seda*DNA data enables to estimate only proportions
314 and NPPs are the most selective with certain species as microfossils.
315



316

317 **Fig. 5.** Sørensen dissimilarity index estimated from composite data set of fossil algae (NPP) and
318 *seda*DNA. Background colors indicate main climatic and environmental changes such as
319 Pleistocene-Holocene boundary, 8.2 ka cooling event and time of agriculture practice at Lake
320 Lielais Svētiņu.

321

322 Based on composite information of algae taxonomy, we reconstructed algae turnover rates (beta
323 diversity in time) for the last 14,500 years. Our estimates indicate higher turnover rates for warmer
324 Holocene period (Fig. 5). During the Lateglacial, only significant shift in algal composition
325 occurred at the beginning of the lake development stages. Otherwise, the end of Pleistocene was
326 secluded from a distinct algal turnover, indicating stable aquatic and terrestrial environment that is
327 in line with the study by Stivrins et al. (2016). The following swing at the boundary of Pleistocene-
328 Holocene occurs when the rate of warming was 0.17 °C/decade that is comparable to the current
329 warming in the Northern Hemisphere (Stivrins et al., 2016; Smith et al., 2015). Relatively low
330 Sørensen dissimilarity indices from 8400 to 7900 cal. BP suggest stable algal composition. This
331 time is known as the 8.2 ka cooling event that led to a drop of mean air temperature in winter by 2–
332 3 °C (Seppä et al., 2007). It seems that algae did not react to this event, but still note high turnover
333 rates right before and after 8.2 ka event. This suggests that algal turnover took place before and
334 after, but during the cooling lake conditions and algal composition was stable in time .

Previous studies from Lake Lielais Svētiņu (Stivrins et al., 2015) and from Lake Højby Sø in Denmark (Hede et al., 2010) indicate that disruption in thermophilous terrestrial vegetation and increased erosional export of nutrients lasted for nearly 700 years. In addition, the 8.2 ka cooling event marks the beginning of increased algae pigment accumulation of Cyanobacteria, Chlorophyta, Cryptophytes and Diatoms that prosper until 5000 cal. BP. Increased abundance of these pigments coincide with the warmest time in Holocene, namely Holocene Thermal Maximum (2.5–3.5 °C above the present day mean temperature; Heikkilä and Seppä, 2010). In our data, algal turnover was low from 6300 to 4700 cal. BP due to flourish of Cyanobacteria indicating prolonged period of water column thermal stratification. Our finding suggests that possible future climate change could shift Lake Lielais Svētiņu trophic state and algae composition back to the state similarly as observed from 6300 to 4700 cal. BP. The last significant increase of algae turnover can be associated with human activities such as agriculture practice (Stivrins et al., 2015) leading to trophic change in lake. However, algae turnover has decreased towards present day that can be explained by decreased population density and agricultural activities around the lake.

While palaeoecological proxies generally reflect changes in landscape, the dynamics in abundance of planktonic NPPs must be viewed with respect to lake's ontogeny (Kisand et al., submitted). Indeed, long-term NPP dynamics probably were dependent from several influencing aspects, such as water level changes of the lake that can be driven also by infilling process (Belle et al., in press) leading to transition from deep to unstratified lacustrine ecosystem.

Conclusions

In the current study, we explored a long-lasting question – how reliable are non-pollen palynomorphs (fossil algae) data, recovered alongside routine pollen analysis. We used *seda*DNA and algal pigment data to validate the richness and abundance of fossil algae. In addition, for the first time, we compiled a composite data-set from fossil and *seda*DNA algae to show how fossil algae can be integrated with other palaeo proxies and estimate algae turnover rates for the last 14,500 years from a small lake Lielais Svētiņu sediments. Our results revealed a mismatch between reconstructed fossil algae accumulation rates between those obtained from algae pigments. As predicted, taxonomically, fossil algae underrepresents species, but still aid those missing from *seda*DNA. Small amount of species were overlapping between fossil and *seda*DNA algae and possible reasons are discussed. Algae turnover rates estimated from a composite data-set indicate lower biotic turnover rates for the Lateglacial (14,500–11,700 cal. BP) and higher for the Holocene (11,700 cal. BP–present). By conducting this study, we encourage a growing number of palynologists keep tracking NPP in their routine work and seek integration possibilities with other ecological and palaeoecological disciplines/proxies in order to tackle important research questions.

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